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BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Paper No. 19

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Appellants: Lynn Bergmeyer
Thomas J. Cummins
John B. Findlay
JoAnne Kerschner

J. Lanny Tucker
For Appellants

EXAMINER'S ANSWER

This is in response to Appellant's brief on appeal filed September 16, 1994.

(1) *Status of claims.*

The statement of the status of claims contained in the brief is correct.

(2) *Status of Amendments After Final.*

The Appellants' statement of the status of amendments after final rejection contained in the brief is correct.

(3) *Summary of invention.*

The summary of invention contained in the brief is correct.

(4) *Issues.*

The Appellant's statement of the issues in the brief is correct at the time the Brief was filed. However, the rejection of Claims 1-8 and 10-38 under Section 112(2) has been overcome by Appellants' arguments in the Brief and is therefore no longer an issue.

(5) *Grouping of claims.*

Claims 1-8, 10-13, 16-20, 22-27, 30, 31, 33, 35-37, and new Claims 39-42 stand or fall together because Appellants' brief does not include a statement that this grouping of claims does not stand or fall together. See 37 C.F.R. § 1.192(c)(5).

(6) *Claims appealed.*

The copy of the appealed claims contained in the Appendix to the brief is correct.

(7) *Prior Art of record.*

The following is a listing of the prior art of record relied upon in the rejection of claims under appeal.

Nedjar et al. (1991) J. Virol. Meth. 35, pp. 297-304;

Brytting et al. (1991) J. Virol. Meth. 32, pp. 127-138;

Gibbs et al. (1990) Genomics 7, pp. 235-244;

Findlay et al. (August 9, 1990) WO 90/08840.

(8) *New prior art.*

Chamberlain et al. (1988) Nuc. Acids Res. 16, pp. 11141-11156.

(9) *Ground of rejection.*

The following ground(s) of rejection are applicable to the appealed claims.

Claims 1-8, 10-13, 16-20, 22-27, 30, 31, 33 and 35-37 are rejected under 35 U.S.C. 103 as being unpatentable over Nedjar et al. in view of Brytting et al., in view of Gibbs et al., and in view of Findlay et al.

Nedjar et al. teaches a PCR method to co-amplify to detect both Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) by a method known as multiplex PCR. The method involves a simultaneous amplification of both HCV, as the first target, and HIV, as the second target, to which two sets of primers are used (page 299, full paragraph). Having succeeded in simultaneous amplification of two distinct viral agents, HCV and HIV, Nedjar et al. indicates that "[t]he ability to amplify and detect HCV and HIV-1 specific genomic sequences simultaneously, by using RNA-PCR, suggests that multiple primer pairs from two different viral agents can be used in a single PCR amplification reaction to identify multiple infections. The number of HCV and HIV-1 nucleic acid copies present in a sample during co-amplification assay does not interfere with the amplification of either sequence." However, not taught by Nedjar *et al.* are the claim

limitations drawn to the sets of primers directed to detect human Cytomegalovirus (hCMV) DNA, as the first target, and a second target DNA which is the same as or different from hCMV DNA; the design of multiple primers which are conformed to similar melting temperature; the presence of thermostable DNA polymerase at at least 10 units/100µl; and diagnostic element comprising a support to which capturing probes are attached.

Brytting et al. teaches primers to detect hCMV DNA (pages 129-131, Figure 1).

Gibbs et al. also teaches a multiplex PCR method, wherein not only two different sets of primers are used to detect two distinct target regions but 8 sets thereof to detect 8 distinct regions on the same target DNA (see Figures 1 and 2). In addition, Gibbs et al. teaches the rationale in the design of these primers: "[a]ll primers were designed to conform as closely as possible to the general formula of 24 bases with a 50% GC content" (page 238, right column, lines 5-7) to achieve simultaneous amplification.

Findlay et al. teaches a nucleic acid test article comprising water-insoluble support to which nucleic acid capture probes are attached (pages 9-21).

It would have been prima facie obvious to one of ordinary skill in the art at the time the instant invention was made to incorporate the teachings presented above to develop the instant method to simultaneously amplify and detect human CMV and other

infectious agent such as HIV. Simultaneous amplification of two distinct viral target and the motivation to do so are taught by Nedjar et al. as presented above. Primers directed to hCMV are taught by Brytting et al. One critical element of the invention is the design of primers which have the same range of melting temperature (T_m) and have T_m's within about 2°C or about 5°C of one another to allow all the primers to function in the same amplification condition. This is taught in the multiplex PCR method by Gibbs *et al.* Indeed, Gibbs et al. teach that all the primers are designed to conform to specific parameters regarding the melting temperature. In fact, to demonstrate this point, a T_m calculation was made by the examiner for the eight oligonucleotide primers of the first four sets (Sense and Antisense primers) taught in Gibbs et al. (Figure 1). These primers are found in the T_m range as specified in the instant claims, i.e., between about 65°C to 74°C and within about 5°C of one another:

First set: 69.1°C, 76.55°C;

Second set: 68.05°C, 68.05°C;

Third set: 66.7°C, 69.41°C;

Fourth set: 65.64°C, 66.69°C.

Other elements of the invention including pH, the concentrations of primers and DNA polymerase, length of primers, additional PCR reagents, capturing probes, and water-insoluble

supports all are taught in the prior art. For instance, Gibbs et al. teaches the concentrations of each primer that range from 0.2μM to 0.5μM (calculated using the amount of primers and volume of the PCR reaction as indicated on the paragraph bridging pages 236 and 237); Nedjar et al. teaches the use of 1μM of each primer (calculated using the amount of primers and volume of the PCR reaction as indicated in the main full paragraph of page 299); Brytting et al. teaches the use of 0.15μM for each primer of one set and 0.60μM for each primer of the other set (page 131, "PCR with one set of primers" Section).

One of ordinary skill in the art thus would have reasonably to empirically determine the specified parameters or conditions claimed. In addition, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use a diagnostic element which is coated with capturing probe to facilitate the detection of amplified product, as taught by Findlay et al. In a time period where more diseases are found to be caused by bacterial or viral agents, such as CMV and HIV (see the Introduction of Gibbs et al. for instance), the ordinary artisan would have been motivated to detect simultaneously more than one infectious agent (via DNA analysis) using a multiplex PCR method.

As for the diagnostic kit, and diagnostic element claims, it would have been prima facie obvious to the artisan to package the reagents to practice the method into a composition or kit because

Gibbs et al. teaches that "[t[he preparation of premixed multiplex kits was a great advantage for reducing problems of contamination, as it reduced the overall number of manipulations that were necessary" (page 238, last sentence).

(10) *Response to argument.*

Appellants' argument has been fully considered by the Examiner but is not deemed to overcome the rejection. There are several factors which Appellants allege would have overcome the rejection.

First, the claimed invention is drawn to the following: (1) a limited amount of primers directed to hCMV DNA and a second target DNA, which are "matched" in melting temperature; (2) a concentration of DNA polymerase of at least 10 units/100µl; (3) using the same temperature for the annealing and extension during PCR so that only two different temperatures are used (the other temperature is for the denaturing step), i.e. "two-temperature- instead of a "three-temperature" PCR; (4) each PCR cycle is within 120 seconds.

The Examiner agrees with Appellants that Nedjar et al. does not relate to the detection of hCMV DNA. Rather, the reference teaches a simultaneous detection of HCV and HIV. The Examiner regrets the error made in the statement which indicates that both Nedjar et al. and Brytting et al. design primers directed to hCMV; only Brytting does so.

As pointed out in the Ground of Rejection, the concentrations of all of the primers taught by Gibbs et al., Nedjar et al., and Brytting et al. fall within the recited concentration in the claims (see Ground of Rejection). The Examiner disagrees with Appellants that the primers taught by Nedjar et al. do not fall within the claims, with respect to the melting temperature. The T_m's calculated by Appellants from Nedjar et al. (66°C and 65.5°C for HIV primers; 66.5°C and 63.1°C for HCV primers) do fall within the claims, which recites a range of from about 65°C to 74°C, and within about 5°C of each other. That the primers are designed to be "matched," i.e., having the T_m's within the range as recited in the claims, is taught by Gibbs et al. Indeed, Gibbs et al. teaches at least eight primers, used in a multiplex PCR, that fall within the range as recited in the claims, as pointed out in the Ground of Rejection. The Examiner notes that Appellants failed to respond to this teaching.

While Gibbs et al. teaches the use of a DNA polymerase concentration of 8 units per 100µl of reaction mixture for multiplex PCR, the instant invention is drawn to a concentration of at least 10 units per 100µl mixture. However, based on Appellants' disclosure, the latter concentration is not critical for the following reason. On page 19 of the specification, Appellants indicate that the preferred concentration is from about 0.1 to about 50 units per 100µl, and the more preferred

concentration is from about 10 to about 25 units per 100µl. The concentration of DNA polymerase taught by Gibbs et al. would fall within the more preferred concentration. Thus even though the concentration of the DNA polymerase as recited in the claims is not the same as that taught by Gibbs et al., it is not critical for it to be so. Moreover, one skilled in the art at the time of the instant invention, or even prior thereto, would have known to determine empirically the optimal concentration of DNA polymerase for a given multiplex PCR. Design and optimization of PCR require not only primers selection but other parameters including PCR buffer, Mg^2 concentration, cycling parameters, and enzyme concentration (e.g. Taq DNA polymerase).

It would also have been obvious for the ordinary artisan to use the same temperature for the annealing and extension steps in a PCR, and to shorten the time of a PCR cycle, since the melting temperature of all primers are already in the range of the temperature in which DNA polymerase is normally used to polymerize for the extension step, which is from about 68°C to about 72°C as taught by all three cited prior art. As Appellants indicate in the Brief, Brytting et al. teaches a cycle which takes only 90 seconds. Though there is no explicit suggestion in the art that multiple targets can be amplified quickly in the same reaction mixture, it would have been obvious and a routine experimentation for the ordinary artisan to determine if a short

cycle, as taught by Brytting et al., would amplify more than one target, with a reasonable expectation of success.

While the factors of Appellants' argument that are based on the "two-temperature" PCR and a 120-second cycle are relevant to the method claims 22-27 and 30-36, they are not with respect to the compositions, kits, or elements claims. This is so because these factors are not limitations recited in these claims.

This rejection is also applied to the new Claims 39-42, which are the independent forms of now-cancelled Claims 9, 15, 32 and 34, respectively, since the instant rejection originally included them.

(11) *New ground of rejection.*

This examiner's answer contains the following NEW GROUND OF REJECTION.

A. Claim 38 is rejected under 35 U.S.C. 103 as being unpatentable over Brytting et al. in view of Gibbs et al. Claim 38 is drawn to an oligonucleotide directed, among others, human CMV (hCMV) DNA having specific sequences (SEQ ID NOS. 3, 16-18) recited in a Markush group.

Brytting et al. teaches oligonucleotides directed to hCMV DNA (pages 129-131, Figure 1).

Gibbs et al. also teaches a multiplex PCR method, wherein not only two different sets of primers are used to detect two distinct target regions but 8 sets thereof to detect 8 distinct

regions on the same target DNA (see Figures 1 and 2). In addition, Gibbs et al. teaches the rationale in the design of these primers: "[a]ll primers were designed to conform as closely as possible to the general formula of 24 bases with a 50% GC content" (page 238, right column, lines 5-7) to achieve simultaneous amplification.

It would have been prima facie obvious to one of ordinary skill in the art at the time the instant invention was made to arrive at the oligonucleotides directed against hCMV. Brytting et al. provides the motivation for making primers directed against hCMV, which is to detect hCMV. Gibbs et al. provides the teaching and rationale to design primers which are conformed to the narrow range of melting temperature.

B. Claims 1-8, 10-13, 16-20, 22-27, 30, 31, 33 and 35-38 are rejected under 35 U.S.C. 103 as being unpatentable over Nedjar et al. in view of Brytting et al., in view of Gibbs et al., in view of Chamberlain et al., and in view of Findlay et al.

Nedjar et al. teaches a PCR method to co-amplify to detect both HCV and HIV by a method known as multiplex PCR. The method involves a simultaneous amplification of both HCV, as the first target, and HIV, as the second target, to which two sets of primers are used (page 299, full paragraph). Having succeeded in simultaneous amplification of two distinct viral agents, HCV and HIV, Nedjar et al. indicates that "[t]he ability to amplify and

detect HCV and HIV-1 specific genomic sequences simultaneously, by using RNA-PCR, suggests that multiple primer pairs from two different viral agents can be used in a single PCR amplification reaction to identify multiple infections. The number of HCV and HIV-1 nucleic acid copies present in a sample during co-amplification assay does not interfere with the amplification of either sequence." However, not taught by Nedjar *et al.* are the claim limitations: the sets of primers directed to detect human CMV (hCMV) DNA, as the first target, and a second target DNA which is the same as or different from hCMV DNA; the design of multiple primers which are conformed to similar melting temperature; the presence of thermostable DNA polymerase at at least 10 units/100µl; and a diagnostic element comprising a support to which capturing probes are attached.

Brytting et al. teaches primers to detect hCMV DNA (pages 129-131, Figure 1).

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Chamberlain et al. teaches a multiplex PCR, which uses (1) 1μM of each primer; (2) 10 units of Taq DNA polymerase per 100μl of the reaction; and (3) a "two-temperature" PCR, i.e. one temperature is for the denaturing step, and the other, 55°C, is for both annealing and extension steps (see paragraph bridging pages 11142 and 11143).

Findlay et al. teaches a nucleic acid test article comprising water-insoluble support to which nucleic acid capture probes are attached (pages 9-21).

It would have been prima facie obvious to one of ordinary skill in the art at the time the instant invention was made to arrive at the instant invention. The Appellant is referred to the rejection reiterated above made under the cited prior art which does not include Chamberlain et al. This rejection is made to further point out that the use of two-temperature PCR and 10 units of DNA polymerase is taught in the prior art by Chamberlain et al.

(12) *Period of response to new ground of rejection.*

In view of the new ground of rejection, appellant is given a period of TWO MONTHS from the mailing date of this examiner's answer within which to file a reply to any new ground of rejection. Such reply may include any amendment or material appropriate to the new ground of rejection. Prosecution otherwise remains closed. Failure to respond to the new ground

of rejection will result in dismissal of the appeal of the claims so rejected.

CONCLUSION

It is respectfully submitted that the rejection of the appealed claims in this application is correct and proper for the reasons noted above and thus should be affirmed.

Any inquiry concerning this communication or those earlier from the Examiner should be directed to Paul B. Tran, Ph.D. whose telephone number is (703) 308-4040. Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose phone number is (703) 308-0196.

Paper related to this application may be submitted to Group 1800 by facsimile transmission. Papers should be faxed to Group 1800 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703) 305-3014.

PBT

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